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● *Technical Note*

HIGH-INTENSITY FOCUSED ULTRASOUND ABLATION OF *EX VIVO* BOVINE ACHILLES TENDON

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Abstract—Small tears in tendons are a common occurrence in athletes and others involved in strenuous physical activity. Natural healing in damaged tendons can result in disordered regrowth of the underlying collagen matrix of the tendon. These disordered regions are weaker than surrounding ordered regions of normal tendon and are prone to re-injury. Multiple cycles of injury and repair can lead to chronic tendinosis. Current treatment options either are invasive or are relatively ineffective in tendinosis without calcifications. High-intensity focused ultrasound (HIFU) has the potential to treat tendinosis noninvasively. HIFU ablation of tendons is based on a currently-used surgical analog, *viz.*, needle tenotomy. This study tested the ability of HIFU beams to ablate bovine tendons *ex vivo*. Two *ex vivo* animal models were employed: a bare bovine Achilles tendon (deep digital flexor) on an acoustically absorbent rubber pad, and a layered model (chicken breast proximal, bovine Achilles tendon central and a glass plate distal to the transducer). The bare-tendon model enables examination of lesion formation under simple, ideal conditions; the layered model enables detection of possible damage to intervening soft tissue and consideration of the possibly confounding effects of distal bone. In both models, the tissues were degassed in normal phosphate-buffered saline. The bare tendon was brought to 23°C or 37°C before insonification; the layered model was brought to 37°C before insonification. The annular array therapy transducer had an outer diameter of 33 mm, a focal length of 35 mm and a 14-mm diameter central hole to admit a confocal diagnostic transducer. The therapy transducer was excited with a continuous sinusoidal wave at 5.25 MHz to produce nominal *in situ* intensities from 0.23–2.6 kW/cm^2. Insonification times varied from 2–10 s. The focus was set over the range from the proximal tendon surface to 7 mm deep. The angle of incidence ranged from 0° (normal to the tissue surface) to 15°. After insonification, tendons were dissected and photographed, and the dimensions of the lesions were measured. Transmission electron micrographs were obtained from treated and untreated tissue regions. Insonification produced lesions that mimicked the shape of the focal region. When lesions were produced below the proximal tendon surface, no apparent damage to overlying soft tissue was apparent. The low intensities and short durations required for consistent lesion formation, and the relative insensitivity of ablation to small variations in the angle of incidence, highlight the potential of HIFU as a noninvasive treatment option for chronic tendinosis. (E-mail: muratore@rrinyc.org)

Key Words: **High-intensity focused ultrasound, High-intensity therapeutic ultrasound, Focused ultrasound surgery, Tendinopathy, Tendinosis.**

INTRODUCTION

Millions of Americans pursuing physical fitness through sports and other activities are hampered by painful musculoskeletal damage. Among the prominent chronic conditions is tendinopathy, especially to the rotator cuff, elbow extensor and Achilles tendon. Tendinopathy affects Americans trying to keep fit, arises from repetitive stress of physical labor (Stenlund et al. 1993) and keeps professional athletes from successfully competing (Kettunen et al. 2006). Recent histological studies have identified tendinopathy (chronic tendon pain) with tendinosis (local degeneration of a tendon) (Warden 2007).

The length and cross-linking of the component collagen fibrils are responsible for the strength of the tendon under tensile loading (Silver et al. 2003, Vanderby and Provenzano 2003). In the early stages of

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tendinosis, the patient is symptom free and unlikely to recognize the need for intervention. Amorphous repair at the site of the small fiber tears grows thick, randomly oriented new fibers, weakening the site (McShane et al. 2006) and increasing the likelihood of further injury in the same location. Multiple cycles of injury and amorphous repair weaken a large region of the tendon (McShane et al. 2006), increasing the likelihood of new injuries that are large enough to cause pain and restrict motion.

Tendinosis can be diagnosed with ultrasound. Linear diagnostic arrays operating near a 10-MHz central frequency are preferred. In a healthy tendon, ultrasonic B-mode video images made using these probes display regular, alternating hyperechoic and hypoechoic bands. The bands are a characteristic of scattering from the underlying fibers when they are arranged parallel to the long (azimuthal) axis of the linear array. The band structure is considerably reduced in tendinosis-afflicted regions, *i.e.*, where the fibers are disordered. Power Doppler ultrasound also is used to image fluid flow in the affected tendons. Vascularity in normal tendons is minimal; thus, obvious vascularities indicate abnormal fluid flow associated with the inflammatory properties of tendinosis (Ohberg and Alfredson 2002).

Treatment modalities for tendinosis include local injections of steroidal and anesthetic drugs (McShane et al. 2006), percutaneous tenotomy by blade (Maffulli et al. 1997) and by needle (McShane et al. 2006), physical therapy (Christenson 2007) and extracorporeal shock wave therapy (ESWT) (Seil et al. 2006).

In percutaneous tenotomy, the poorly-formed tendon fibers are damaged (fenestrated) deliberately by the therapist, to break them up and induce bleeding, and also to break up calcifications that might be present (McShane et al. 2006). Percutaneous tenotomy by blade is effective in treating certain damaged regions that are accessible to the surgical blade (Maffulli et al. 1997). However, incision into overlying skin and tissues requires skin closure such as suturing. Thus, percutaneous tenotomy by blade is a minor surgical procedure that presents associated risks and requires local anesthetic and sterile surgical conditions.

Percutaneous tenotomy by needle is somewhat less invasive than tenotomy by blade and is suitable for treating less-accessible tendons. A minor skin puncture is made and some needle-track damage occurs to tissues overlying the area to be treated. However, the needle provides considerable tactile feedback to the caregiver. With additional guidance from B-mode ultrasound, the caregiver can break up calcifications. Patients sometimes are unwilling to submit to blade and needle tenotomy procedures.

Treatments sometimes are combined. For example, the tenotomy needle typically is used to inject steroid and local anesthetic in conjunction with fenestration. The procedure is often followed by physical therapy.

ESWT is a widely-used, noninvasive, ultrasonic alternative to percutaneous tenotomy. The FDA has approved ESWT for tennis elbow (lateral epicondylitis) treatments. Despite its success to date, ESWT has some limitations in treating tendinosis. The ESWT focal region is broad; Cleveland et al. (1998) report an effective typical *in vivo* focal region size of 82×20 mm^2. From shock to shock, the focal region wanders and there is a potential for damage to nearby bone (Furia 2006). ESWT

Fig. 1. A 5-annulus Sonic Concepts therapeutic ultrasound transducer was used to ablate bovine tendon *ex vivo*. Outer diameter was 33 mm and focal length was 35 mm when the elements were in phase. Central frequency was 5.25 MHz and −12 dB bandwidth was 45%. A central 14-mm diameter hole admitted a B-K Medical diagnostic array transducer for aiming and monitoring. The transducers are arranged coaxially and confocally. The initial *ex vivo* tissue model is shown with an unsheathed bovine Achilles tendon pinned to a rubber backing and immersed in PBS. In another set of experiments, the tendon and rubber target were replaced with a layered chicken breast, bovine tendon and glass target.

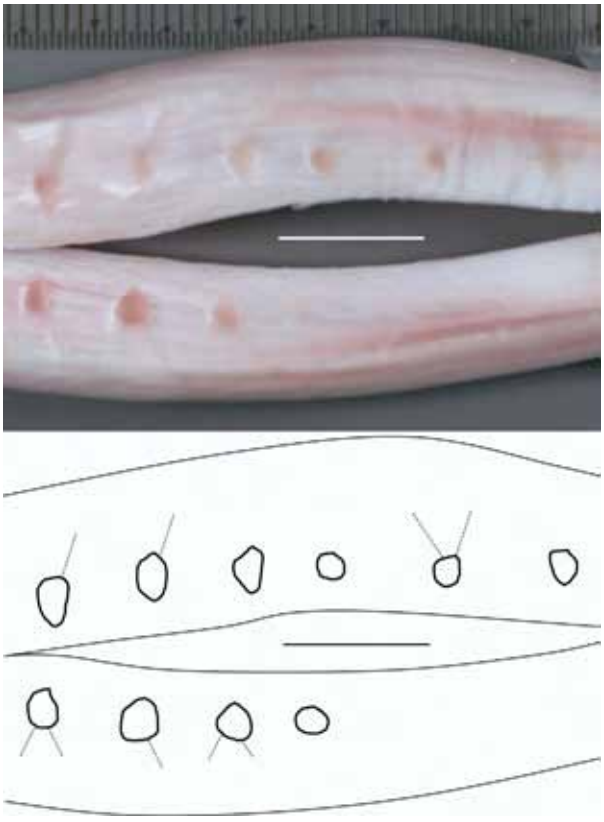

Fig. 2. Six HIFU lesions were made in an unsheathed bovine Achilles tendon at 23°C, with a therapeutic transducer excited at a frequency of 5.25 MHz for 5 s, with an *in situ* intensity of 0.55 kW/cm^2. To reveal the lesions after ablation, the tendon was sliced longitudinally and the two halves folded outward. The surface of the bovine tendon distal to the therapeutic transducer is central in the image. Lesions appear as darker regions. The horizontal bar is 10 mm long. Lesion dimensions are tabulated in Table 1. The image was color-corrected with Adobe Camera Raw. In the schematic, the focal lesions are indicated with dark solid lines and the slight ablation tracks along the beam path are indicated with dotted lines. The cleavage process missed the two rightmost lesions; hence they are not seen on both sides of the split. Tissue was scraped away to reveal their extent.

is more effective when calcifications are the root cause of pain and less effective when little or no calcific involvement is present (Harniman et al. 2004). ESWT is difficult to aim; currently popular machines in the United States, such as the SONOCUR (Siemens Medical Solutions USA, Inc., Iselin, NJ, USA), are aimed only by pointing the shock wave head at an external anatomical landmark (Cleveland et al. 1998). These limitations of ESWT suggest the need for further development of additional, complementary treatments for tendinosis.

The well-known therapeutic bioeffects of high-intensity focused ultrasound (HIFU), also known as focused ultrasound surgery (FUS), include thermal and cavitational mechanisms (Harvey 1930). A considerable amount of work is ongoing in HIFU. Commercial devices that use HIFU to ablate a variety of tissues are now marketed worldwide.

HIFU has the potential to overcome some of the limitations of ESWT and percutaneous tenotomy while retaining some of their key features. Like percutaneous needle tenotomy, HIFU is used under ultrasound guidance, has controllable targeting and has the potential to be applied rapidly with a hand-held transducer. Like ESWT, it is noninvasive.

It has long been known (Coleman et al. 1985) that HIFU can damage collagen fibers and stimulate subsequent collagen growth in the sclera, as seen 2–4 weeks post insonification by increases in the number of fibroblasts and in collagen fibril thickness.

It is anticipated that HIFU is a potential means to facilitate tendon healing, because of (i) the efficacy of the surgical analog; (ii) the efficacy of ESWT, despite its limitations *vis-à-vis* focus; and (iii) studies of scleral collagen showing a stimulus in collagen fibril production after HIFU ablation.

Because of the high acoustic attenuation coefficient of tendon (Goss et al. 1979), the ability of HIFU to ablate regions of therapeutic interest millimeters deep in tendon remains to be quantitatively characterized. In this study, evidence is presented showing that HIFU can ablate tendons *ex vivo*.

MATERIALS AND METHODS

Ablation

This study used two distinct *ex vivo* tissue models. An initial set of trials, designed to identify effective exposure parameters under simple conditions, featured an unsheathed bovine Achilles' tendon (deep digital flexor) stabilized on a rubber backing (Fig. 1). A second set of trials, designed to more accurately simulate the treatment of tendon *in vivo*, featured a layered model. Chicken breast muscle was sliced into sections 8–10 mm thick. A chicken slice was placed over the unsheathed bovine tendon (proximal to the transducer). The presence of the proximal muscle tissue enabled assessment of potential collateral damage to overlying tissue. A glass plate was placed under the tendon (distal to the transducer) to simulate the highly echogenic bone often present near tendons (Moros 2004).

Tissue samples were obtained from local butchers, trimmed to size, immersed and degassed in normal phosphate-buffered saline (PBS) and maintained at 23°C or 37°C (± 0.1°C) during insonification.

The therapeutic ultrasound transducer (Sonic Concepts, Inc., Bothell, WA, USA) (Muratore et al. 2005) was a 5-annulus array with an outer diameter of 33 mm,

Table 1. Lesion dimensions were determined visually for the HIFU *ex vivo* ablated bovine Achilles tendon shown in Fig. 2

Model	Temperature (°C)	Intensity (kW/cm^2)	Time (s)	Depth (mm)	Angle (°)	Length (mm)	Width (mm)
Bare tendon	23	0.55	5	6	0	3.60	1.92
Bare tendon	23	0.55	5	6	0	3.60	2.52
Bare tendon	23	0.55	5	6	0	2.88	1.80
Bare tendon	23	0.55	5	6	0	2.76	1.68
Bare tendon	23	0.55	5	6	0	2.28	1.20
Bare tendon	23	0.55	5	6	0	2.76	2.04
					Mean	2.980	1.860
					SD	0.523	0.434

The HIFU transducer was excited at 5.25 MHz. Temperature is that of the PBS bath in which the sample was immersed during insonification. Intensity is the *in situ* intensity estimated by assuming acoustic attenuation of 2.9 dB/(MHz · cm) in tendon. Time is the duration of insonification. Depth is the position of the nominal transducer focus below the proximal tissue surface. Angle is the angle of incidence of the acoustic beam; 0° is normal to the tissue surface. Length is the lesion extent along the beam axis. Width is the lesion extent transverse to the beam axis.

an unphased focal length of 35 mm and a central 14-mm diameter hole that admitted a confocal diagnostic transducer (Fig. 1). The therapeutic transducer had a center frequency of 5.25 MHz and a –12 dB bandwidth of 45%. Using the cylindrically symmetric solution of O'Neil (1949), the half-power focal region in water was calculated to be 0.28 mm in diameter and 2.5 mm long axially. Acoustic power, measured before each therapy session with a flat absorbing target wattmeter (Muratore 2006), varied from 7.0–9.3 W. Assuming an absorption coefficient of 0.5 dB/(MHz · cm) in chicken and 2.9 dB/(MHz · cm) in tendon (Goss et al. 1979), the *in-situ* focal-region intensities corresponding to the measured power range were estimated to be 0.55–0.90 kW/cm^2 in the bare-tendon model and 0.23–2.6 kW/cm^2 in the layered model. Duration of insonification varied from 2–20 s. The diagnostic transducer used to aim and monitor ablation was a 48-element linear array (model 8663, B-K Medical, Herlev Hovedstaden, Denmark) with a central frequency of 6.5 MHz and a −12 dB bandwidth of 40%. The therapeutic and diagnostic transducers were joined with a molded collar (visible in Fig. 1), which maintained their relative confocal and coaxial positions. This arrangement permitted the depth of focus of the therapy transducer to be determined from the B-mode display. The nominal focus was set to a depth in the tendon that varied from 0–8 mm. Angle of incidence varied from 0° (normal to the tissue surface) to 15°. Angle and depth adjustments were made *via* a bracket supporting the transducers.

The therapeutic and diagnostic transducers were operated with the HIFU2 integrated therapeutic/diagnostic ultrasound system (Muratore et al. 2004).

Visual characterization of lesions

The tendon was examined visually after insonification. Marker lesions on the tendon surface (proximal to the transducer) identified the line along which lesions were produced. The tendon was sliced along this line to expose a longitudinal plane. Overlying chicken was sliced to expose the same plane.

Images of sliced specimens were obtained with a digital camera (model EOS D60 with EF 28-70mm f/2.8L USM lens, Canon U.S.A., Inc., Lake Success, NY, USA). Photographs were color-corrected using a white-balance standard in Camera Raw software (version 4.1, Adobe Systems, Inc., San Jose, CA, USA).

In the tendon images, the more intensely–colored regions near the nominal beam focus were identified as lesions. In the chicken, the less intensely–colored re-

Table 2. Lesion dimensions determined visually for HIFU *ex vivo* ablated bovine Achilles tendon

Model	Temperature (°C)	Intensity (kW/cm^2)	Time (s)	Depth (mm)	Angle (°)	Length (mm)	Width (mm)
Bare tendon	37	0.90	2	5	0	3.56	1.64
Bare tendon	37	0.90	2	5	0	4.39	1.79
Bare tendon	37	0.90	2	5	0	4.03	1.51
Bare tendon	37	0.90	2	5	0	3.20	2.10
Bare tendon	37	0.90	2	5	0	3.72	1.67
Bare tendon	37	0.90	2	5	0	3.10	2.29
					Mean	3.667	1.833
					SD	0.492	0.300

Parameters are as defined in the legend for Table 1.

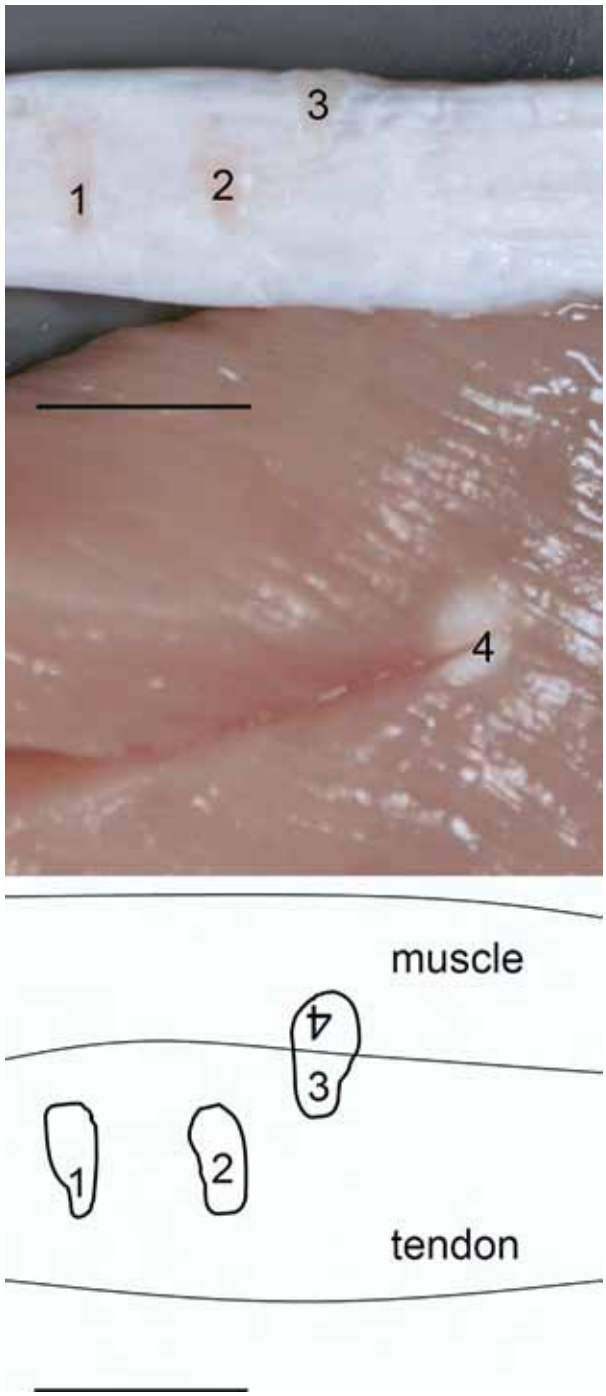

Fig. 3. HIFU lesions were made in the layered model (chicken breast, bovine Achilles tendon, glass). The therapeutic transducer was located above the figure with the ultrasound beam propagating down. The schematic demonstrates the configuration before the tissue was rearranged to reveal the lesions. After ablation, the chicken breast was folded down and the chicken and tendon were sliced longitudinally. The lighter-colored tendon appears at the top and is shown in cross-section. The darker-colored chicken breast appears at the bottom and is oriented so that the surface that was adjacent to the tendon during ablation faces the viewer. The horizontal bar is 10 mm long. The darker regions labeled 1 and 2 are intramural lesions made 6 mm below the surface of the tendon, at a frequency of 5.25 MHz, an exposure of 10 s and an *in situ* intensity of 0.32 kW/cm^2. The darker region labeled 3 is a lesion made at the proximal tendon surface at a frequency of 5.25 MHz, an exposure of 10 s and an *in situ* intensity of 2.6 kW/cm^2. No soft tissue ablation accompanied the subsurface lesions. However, the lighter region labeled 4 is a lesion made simultaneously with and adjacent to superficial lesion 3. The image was color-corrected with Adobe Camera Raw.

gions were identified as lesions. Lesion width (transverse extent of the region relative to the beam propagation direction) and length (axial extent) were obtained from the images. Dimensions were calibrated with a millimeter scale within the field-of-view.

Histological characterization of lesions

Samples of untreated and treated regions of the 37°C bare-tendon model were placed in 10% glutaraldehyde diluted to 2.5% with normal PBS, secondarily fixed with osmium tetroxide-potassium ferricyanide, *en bloc* stained with uranyl acetate, dehydrated through a graded series of ethanols and embedded in Spurr's resin and sectioned at 65 nm. Sections were further contrasted with lead citrate. Transmission electron micrographs were obtained at 4800x on Kodak 4489 EM film (Eastman Kodak Co., Rochester, NY, USA). Photographic negatives were scanned; subsequent images were cropped and resized in Photoshop software (version CS3, Adobe Systems, Inc.).

RESULTS

Figure 2 shows six HIFU lesions created at a depth of 6 mm in the bare-tendon model at 23°C, with an *in situ* intensity of 0.55 kW/cm^2 and a 5-s insonification. Some slight evidence is seen of ablation along the therapeutic beam track for three of the lesions; the tracks were not included in the lesion sizes. The mean lesion length was 2.98 mm (standard deviation = 0.523, 6 samples), and the mean lesion width was 1.86 mm (standard deviation = 0.434, 6 samples). The individual lesion dimensions are tabulated in Table 1.

Six additional HIFU lesions were created at a depth of 5 mm in the bare-tendon model at 37°C, with an *in situ* intensity of 0.90 kW/cm^2 and a 2-s insonification. These lesions were nearly cylindrical in shape. The mean lesion length was 3.667 mm (standard deviation = 0.492, 6 samples), and the mean lesion width was 1.833 mm (standard deviation = 0.300, 6 samples). The individual lesion dimensions are tabulated in Table 2.

In the transmission electron microscope images of the longitudinally-sectioned bovine tendon (Fig. 4), un-

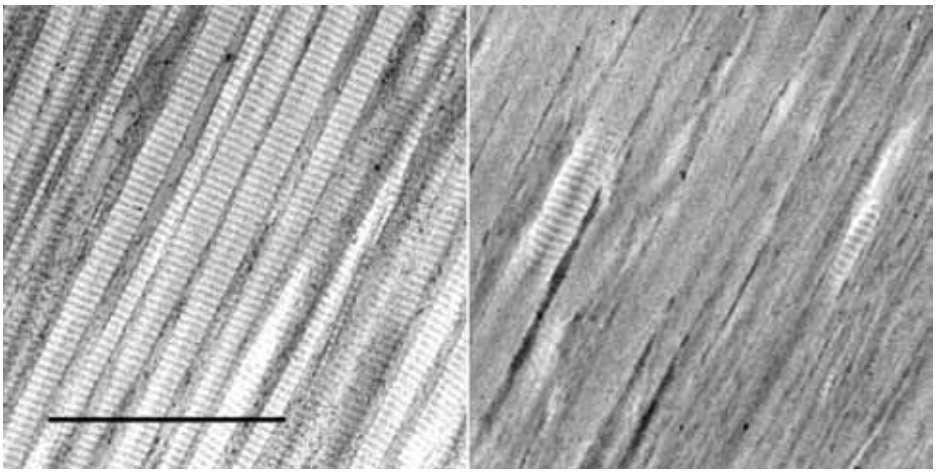

Fig. 4. Transmission electron micrographs of longitudinally-sectioned bovine tendon (obtained at 4,800x). The horizontal black bar is 1 μm long. (*Left*) Normal collagen tissue exhibits characteristic striping of collagen fibrils throughout the sample. (*Right*) HIFU-ablated collagen tissue exhibits striping only in isolated areas.

treated collagen tissue exhibits characteristic striping of collagen fibrils throughout the sample; HIFU-ablated collagen tissue exhibits striping in isolated areas only.

In the layered model, subsurface intramural lesions (*e.g.*, lesions 1 and 2 in Fig. 3) in the tendon did not cause visible damage in the overlying soft tissue. The distal hard glass surface apparently did not interfere with the ability to produce intramural lesions. Lesions aimed at the interface between the tendon and the overlying soft tissue formed adjacent lesions in both tissues (*e.g.*, lesions 3 and 4 in Fig. 3).

A 15° increase in the angle of incidence produced shorter, fatter lesions. As listed in the upper half of Table 3, lesions produced with normal beam incidence (0°) had a mean length of 5.92 mm (standard deviation = 0.524, 6 samples) and a mean width of 2.19 mm (standard deviation = 0.621, 6 samples), whereas lesions produced with a beam incidence of 15° had a mean length of 3.97 mm (standard deviation = 0.181, 4 samples) and a mean width of 2.76 mm (standard deviation = 0.272, 4 samples).

A 30% variation in intensity had no significant effect on lesion dimension in the layered model. As listed in the lower half of Table 3, at an *in situ* intensity of 0.34 kW/cm^2, the mean lesion length was 4.48 mm (3 samples) and the mean lesion width was 2.32 mm (3 samples). At an *in situ* intensity of 0.26 kW/cm^2, the mean lesion length was 4.51 mm (2 samples) and the mean lesion width was 1.86 mm (2 samples).

DISCUSSION/SUMMARY

Making thermal lesions in tendons using HIFU is possible under *ex vivo* conditions at 23°C and 37°C.

Common first aid treatment for athletic injuries is "PRICE" (protection, rest, ice, compression, and elevation) (Bleakley 2007). Many tendons of interest lie near the body surface and are thus typically cooler than core body temperature. The application of ice packs to the damaged areas further cools the tendons. The room temperature experiments demonstrate that HIFU (which is very sensitive to ambient temperature) is capable of

Table 3. Lesion dimensions determined visually for HIFU *ex vivo* ablated bovine Achilles tendon in the layered (chicken, tendon, glass) model

Model	Temperature (°C)	Intensity (kW/cm^2)	Time (s)	Depth (mm)	Angle (°)	Length (mm)	Width (mm)	Number
Layered	37	0.23	10	7	15	3.97	2.76	4
Layered	37	0.25	10	7	5	3.46	2.31	6
Layered	37	0.25	10	7	0	5.92	2.19	6
Layered	37	0.26	10	6	0	4.51	1.86	2
Layered	37	0.32	10	6	0	4.07	2.06	1
Layered	37	0.34	10	6	0	4.48	2.32	3

Dimensions are averages; the number of measurements used to compute the averages are indicated in the far right column. All other parameters are as defined in the legend for Table 1. In the upper group, angle of incidence was varied; lesion depth was measured along the beam track. In the lower group, intensity was varied.

producing controlled lesions in tendons at a temperature lower than core body.

Subsurface ablation of tendons does not appear to damage overlying soft tissue *ex vivo*. The presence of a glass plate beneath the tendon did not impede the ability to produce intramural lesions. The production of lesions nearer to a tendon/bone interface is planned for future studies.

Adjunct therapies in a future clinical setting can be anticipated, in which HIFU ablation is combined with low-intensity therapeutic ultrasound for the enhancement of local blood flow (Warden and McMeeken 2002, Demmink et al. 2003), angiogenesis (Young and Dyson 1990), soft-tissue healing (Giombini et al. 2006), the treatment of the tendon-bone interface (Wang et al. 1994, Lu et al. 2006, Walsh et al. 2007) or the acceleration of bone fracture healing (Heckman et al. 1994). Such treatment combinations would benefit from consideration of the underlying cellular mechanisms of tendon damage and ultrasound therapy. The cellular and molecular processes accompanying collagen damage are beginning to be understood (Arnoczky et al. 2007). In parallel, there is considerable research into the activation of cellular mechanisms by HIFU; increasing dose leads to gene activation (Liu et al. 2006) and associated apoptosis (Luo et al. 2007, Yumita et al. 2008), protein denaturation and cell lysis. The loss of the 67-nm characteristic striping of tropocollagen (Fig. 4) suggests that protein denaturation plays a role in HIFU lesion formation in tendon under the conditions presented here.

The *ex vivo* lesions formed in this study were made with consistent intramural locations and dimensions in thick tendon specimens. The frequency, intensity and time required to create the lesions are convenient for possible future clinical applications (although higher power or longer exposure times might be required *in vivo*). The consistency of the observed lesion dimensions and locations and the insensitivity of the method to small variations of the angle of incidence and of the *in situ* intensity suggest that thermal HIFU is a robust technique with promise as a future clinical means of treating tendinosis.

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